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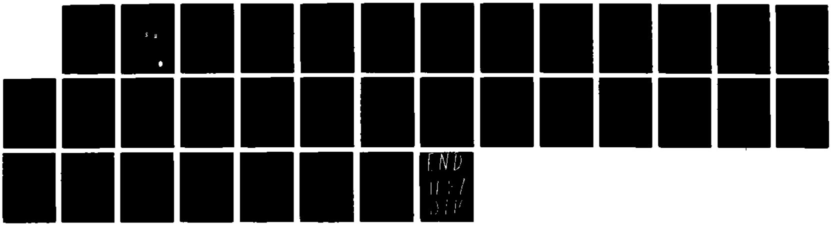

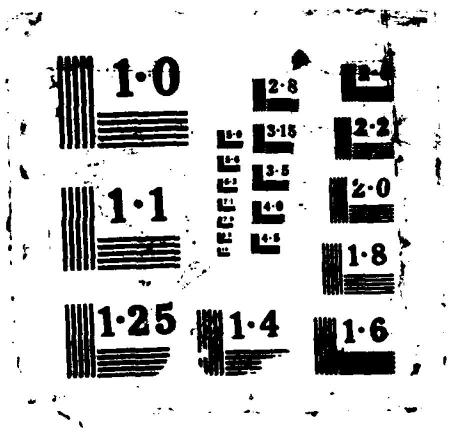
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RADIOFREQUENCY RADIATION AND CELLULAR SECRETORY PROCESSES

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The purpose of this study was to investigate the effect of unmodulated 915-MHz radiation on the secretory processes of exocrine and endocrine cells under in vitro conditions. Pancreatic tissue slices were exposed to 915-MHz radiation in a Crawford cell exposure system. Sham-exposed slices were either incubated at 37 °C or 40 °C. These slices were pulse-labeled with tritiated L-leucine and the labeled secretory proteins were determined by autoradiography and counting of silver grains. In another experiment, amylase secretion from exposed and sham-exposed pancreatic slices was determined. The results suggest that radiation exposure can alter exocrine and endocrine secretions under conditions where the microwave energy produced hyperthermia in localized areas.

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RADIOFREQUENCY RADIATION AND CELLULAR SECRETORY PROCESSES

Introduction

The efforts of government, industry, and medicine today are increasing the applications and use of electromagnetic radiation in the microwave range. When microwaves travel through a human individual, some of the energy is absorbed by the individual's body. It is well known that the absorbed energy increases the rotational and vibrational energies of molecules, and is then dissipated as heat. Some investigators believe that microwave interaction with a human's tissues can also produce nonthermal field effects. Microwave interaction with human tissues thus has the potential of affecting human health by both thermal and nonthermal means.

The purpose of this study was to investigate the effect of unmodulated 915-MHz radiation on the secretory processes of exocrine and endocrine cells under in vitro conditions. Most of the research focused upon the microwave interaction with rat pancreatic tissue slices, and the effects of this interaction on the intracellular transport, packaging, and secretion of the tissue's digestive enzymes. Some experiments were also conducted to investigate the effect of the 915-MHz radiation on the secretion of polypeptide hormones by rat anterior pituitary slices.

Since the exocrine pancreas has served as a model system for studying the secretory processes of granule-bearing cells, we have used it as a model system for investigating the effects of microwaves on glandular secretions. On a mass basis, the pancreas consists almost exclusively of (1) exocrine acinar cells and (2) the ductal system which conducts the secretions of the exocrine cells. The exocrine acinar cells all secrete a common set of digestive enzymes in constant proportions under both control and cholinergically stimulated conditions (the cells were maximally stimulated to secrete in our experiments with 20 μM carbamylcholine chloride (CC)). The kinetics of the intracellular transport and packaging of the digestive enzymes and their overall rate of secretion from the cells can be examined by following the fate of pulse-labelled proteins in the tissue slices (in our studies, the tissue slices were pulse-labelled with tritium-labelled L-leucine). The overall rate of secretion from the cells can also be determined by measuring the rate at which the activity of one of the digestive enzymes increases in the medium bathing the tissue slices (in our studies, we measured the rate of secretion of amylase activity).

The processes by which pancreatic exocrine cells produce digestive enzymes for secretion can be divided into the following steps:

(1) synthesis of the enzymes by the ribosomes of the rough endoplasmic reticulum (ER),
(2) transport of the enzymes from the rough ER to the Golgi complex,
(3) packaging of the enzymes as they are transported through the Golgi complex,
(4) storage of the enzymes in mature zymogen granules, and

(5) exocytotic discharge of the enzymes.

Electron microscope autoradiographic studies have established the time course of the intracellular transport and packaging steps (3-6). When pancreatic exocrine cells are pulse-labelled with radioactive L-leucine for 3 min, 86% of the incorporated radioactivity is located within the cisternae of the rough ER at the end of the 3-min pulse period. At 10 min after the pulse period, almost equal amounts of radioactivity (representing about 45% of the incorporated radioactivity) are found in the rough ER and Golgi complex. At 20 and 40 min after the pulse period, 58% and 64%, respectively, of the radioactivity resides within the Golgi complex; radioactivity in the zymogen granules increases from 3% to 11%. From 60 to 120 min after the pulse period, the percentage of radioactivity within the Golgi complex decreases from 47% to 12% as the percentage of radioactivity in zymogen granules increases from 33% to 59%. The time course of this transfer of radiolabelled secretory proteins from the rough ER to the Golgi complex and then into zymogen granules does not vary with the extent to which the cells are stimulated to secrete.

Evidently, experiments which measure the rate of amylase secretion measure exclusively the rate of the last of the just mentioned steps (i.e., the rate of the exocytotic discharge of the enzymes). By contrast, pulse-labelling experiments can be used to measure the rate of all except the first of the just mentioned steps. However, in our studies, pulse-labelling experiments could measure the rates of only the last three steps; because the time required to pulse-label and wash the tissue slices before placing the slices in a chamber for radiation exposure spans the time during which the pulse-labelled proteins are transported from the rough ER to the Golgi complex.

MATERIALS AND METHODS

Preparation of Pancreatic Tissue Slices

Sprague-Dawley rats (weighing 150-200 g) were fasted for 12-16 h before decapitation. The pancreas was excised and immediately immersed in ice-cold medium I (118.8 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, 5.6 mM D-glucose, and 0.00001% phenol red). After fat and mesentery were trimmed away, the tissue was cut with a razor blade into slices with dimensions of 1 x 1 x 3 mm. Tail portions of the gland were selected to minimize the presence of large secretory ducts and blood vessels.

Pulse-labelling and Processing of Pancreatic Tissue Slices for Measurement of Release of Pulse-labelled Secretory Proteins

Freshly prepared tissue slices were incubated at 37 °C in 10 ml fresh medium I for 10 min to deplete intracellular stores of L-leucine. The medium was then replaced with 3 ml medium I containing 25 µCi tritium-labelled L-leucine, and the tissue slices incubated for 5 min. The tissue slices were then washed 3 times with 10 ml medium II (105.4 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, 5.6 mM D-glucose, 2 mM L-glutamine, 4.9 mM sodium pyruvate, 5.4 mM sodium fumarate, 0.00001% phenol red, and 1X Eagle's essential and nonessential amino acids) so as to reduce the specific radioactivity of intracellular L-leucine and thereby terminate the radiolabelling of secretory proteins. Three to four tissue slices were then placed in individual T-flasks containing 3 ml medium II and gassed for 10-15 s with 5% carbon dioxide:95% oxygen before postpulse incubation.

After 30-, 90-, 150-, and 210- min postpulse incubation, 1.0 ml aliquots of medium were collected. The aliquots were replaced with 1.0 ml fresh medium II at the first 3 time points. The 1.0 ml aliquots of media were mixed with 0.5 ml ice-cold 2% (w/v) bovine serum albumin and 0.5 ml ice-cold 40% (w/v) trichloroacetic acid (TCA). The TCA-precipitable radioactivity was used to measure the release of pulse-labelled secretory proteins. The tissue slices were processed for radioactivity determination by homogenization in 2.0 ml 0.1% (w/v) sodium dodecyl sulfate (SDS) and 0.1 N NaOH.

The release of pulse-labelled secretory proteins was expressed on both a cumulative and periodic basis. Cumulative percent release expresses the percentage of incorporated radioactivity released after 30, 90, 150, or 210 min postpulse incubation; periodic percent release expresses the percentage of incorporated radioactivity released during just the 0-30, 30-90, 90-150, or 150-210 min postpulse period. Some results are expressed on a periodic basis because statistical analysis shows that microwave radiation can affect the release of pulse-labelled secretory proteins during certain postpulse periods.

Pulse-labelling and Processing of Pancreatic Tissue Slices for Analysis of the Kinetics of the Intracellular Transport of Pulse-labelled Secretory Proteins

The pulse-labelling protocol just described was followed except that the tissue slices were incubated in 3 ml medium I containing 200 μCi tritium-labelled L-leucine for first 10 min at 0 °C and then 3 min at 37 °C. The 10- min incubation at ice-bath temperature permits maximal radiolabelling of the intracellular L-leucine pool in the absence of any detectable incorporation into proteins.

The pulse-labelled tissue slices were incubated in 25- cm^2 T-flasks containing 3.0 ml medium II. After 10-, 20-, 40-, and 60- min incubation, the tissue slices were removed and fixed in phosphate-buffered 3% glutaraldehyde. After postfixation in 2% osmium tetroxide, tissue slices were dehydrated through a graded series of ethanol solutions and embedded in Araldite epoxy resin.

Thin sections were exposed to Ilford L2 track emulsion for 2-4 months. The emulsion was processed for compact grain development after intensification of the latent grains with gold thiocyanate. Sections were observed and photographed using a JEOL 100-S electron microscope; all photographs for statistical analysis of grain location were taken at a magnification of 10,000 and printed at a final magnification of 25,000. Radioautographs were analyzed by the method of Nadler (7).

Incubation of Pancreatic Tissue Slices for Measurement of Amylase Release

Freshly prepared tissue slices were incubated in medium II and aliquots of the medium removed after 15-, 75-, 135-, and 195- min incubation; all aliquots were kept at 0 °C until assayed for amylase activity. After incubation, tissue slices were homogenized in ice-cold amylase buffer. All samples were assayed by the method of Bernfeld (1). Amylase activity was defined as milligrams of maltose generated in 10 min at 30 °C. The results were expressed as the percentage of total amylase activity in the tissue slices released at the 4 time points.

The time points selected for measurement of amylase release were different from those selected for measurement of pulse-labelled secretory protein release because, in pulse-labelling experiments, 15 min were required to pulse label and rinse the tissue slices, place the slices in flasks, and then distribute the flasks among the incubation chambers. When the medium is removed from the pulse-labelled tissue slices 30 min after the beginning of the pulse-labelling period, the tissue slices are (in effect) heated and/or exposed to radiation for only 15 min Consequently, amylase release from heated and/or exposed tissue slices is measured first after 15 min incubation, and then at 1-h intervals thereafter.

Incubation Conditions

Tissue slices were incubated in all experiments in 25- cm^2 T-flasks maintained in warm-air chambers. The slices were exposed to an unmodulated 915-MHz signal in a Crawford Cell Transmission Electron Microscope (TEM) Test Chamber (Model CC110, Instruments for Industry, Inc., Farmingdale, New York). The test chamber was capped with a coaxial termination to eliminate any significant signal reflection.

In individual experiments, tissue slices were exposed to a power density of 5, 10, or 25 mW/cm^2. Measurements of the specific absorption rate (SAR) were conducted at the Public Health Service Food and Drug Administration Bureau of Radiological Health laboratories (Rockville, Maryland) under the guidance of Mr. Stewart Allen. Changes in temperature as a function of time were determined using two Vitek model 101 electrothermia monitors, which were read with a Hewlett Packard (HP) model 3497A data acquisition system and an HP model 1000 computer. Baseline temperatures were established for 30 min before application of electromagnetic energy.

Figure 1 shows the orientation of the T-flasks in the test chamber when tissue slices were exposed to microwaves at power densities of 5 and 10 mW/cm^2; the SAR was less than 0.02 mW/g, and no temperature increase could be recorded under these exposure conditions. To secure a measurable SAR and temperature increase, the configuration of the T-flasks in the test chamber was changed (Fig. 2) and the power density increased to 25 mW/cm^2. Under these conditions, an SAR of 14.5 mW/g was determined and a 3 °C increase in temperature was recorded. With the equipment available to us, we had to use T-flasks as containers of the tissue slices to obtain a measurable SAR.

In experiments conducted with a power density of 25 mW/cm^2, one T-flask containing nonstimulated tissue slices and one T-flask containing slices stimulated with 20- µM carbamylcholine were placed under each of the following conditions:

(1) Irradiated condition (microwave-heated condition): The flasks were placed in a T-cell chamber located in a 37 °C egg incubator; an electromagnetic field was generated in the T-cell chamber.

(2) The 37 °C control: The flasks were placed in a T-cell chamber located in a 37 °C egg incubator; no electromagnetic field was applied.

(3) The 40 °C control (kinetically heated condition): The flasks were placed in a 40 °C tissue culture incubator. The 37 °C control and the 40 °C control conditions were similar in that, under both conditions, the T-flasks were being warm-air-heated.

Control measurements established that the 3 °C increase in temperature generated under the kinetically heated condition had the SAME kinetics as the 3 °C increase in temperature in the microwave-heated condition (Fig. 3). Consequently, any difference in data recorded between the kinetically and microwave-heated conditions cannot be due to a differential heating effect.

Preparation of Anterior Pituitary Tissue Slices

Rat anterior pituitaries were immersed in medium I at room temperature and sliced with a razor blade into quarters.

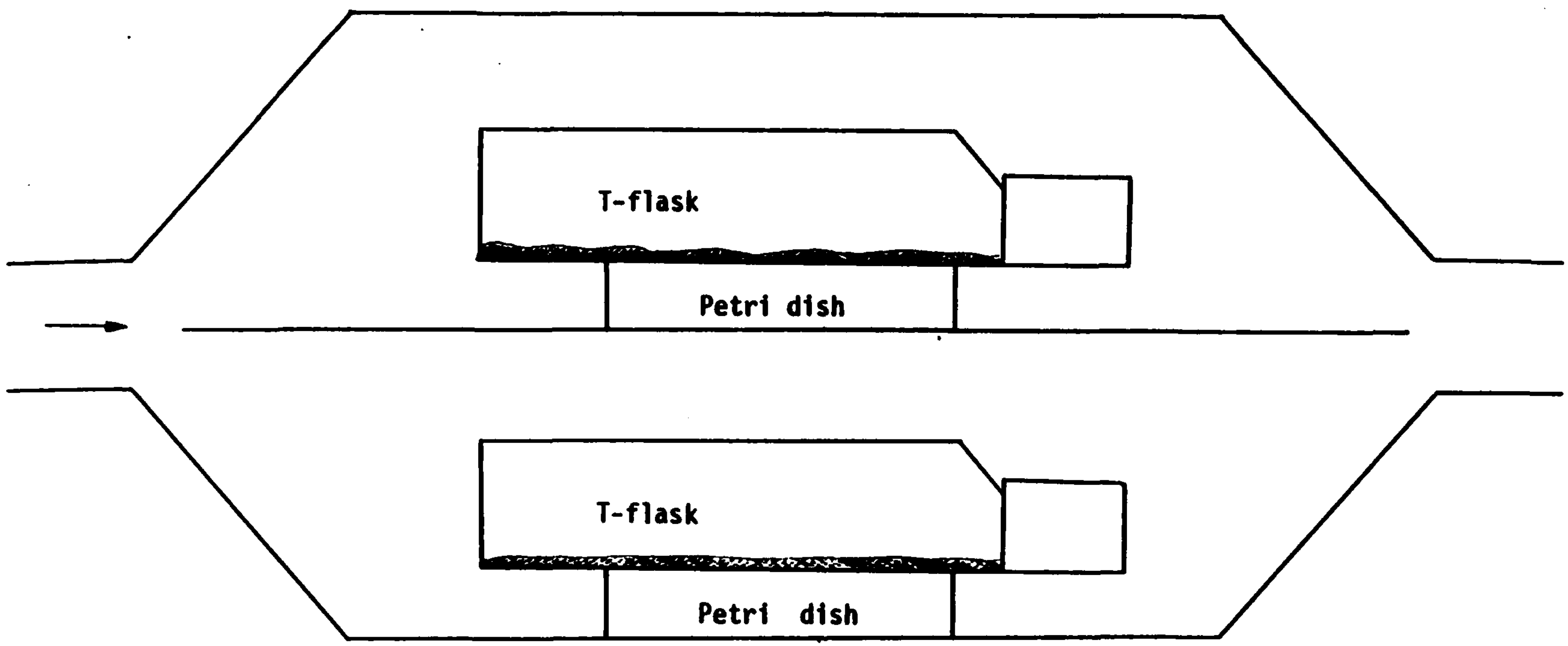

Figure 1. Geometric conformation of T-flasks in the Crawford cell TEM test chamber during experiments with exposure power densities of 5 mW/cm^2 and 10 mW/cm^2. The arrow denotes the direction of energy flow.

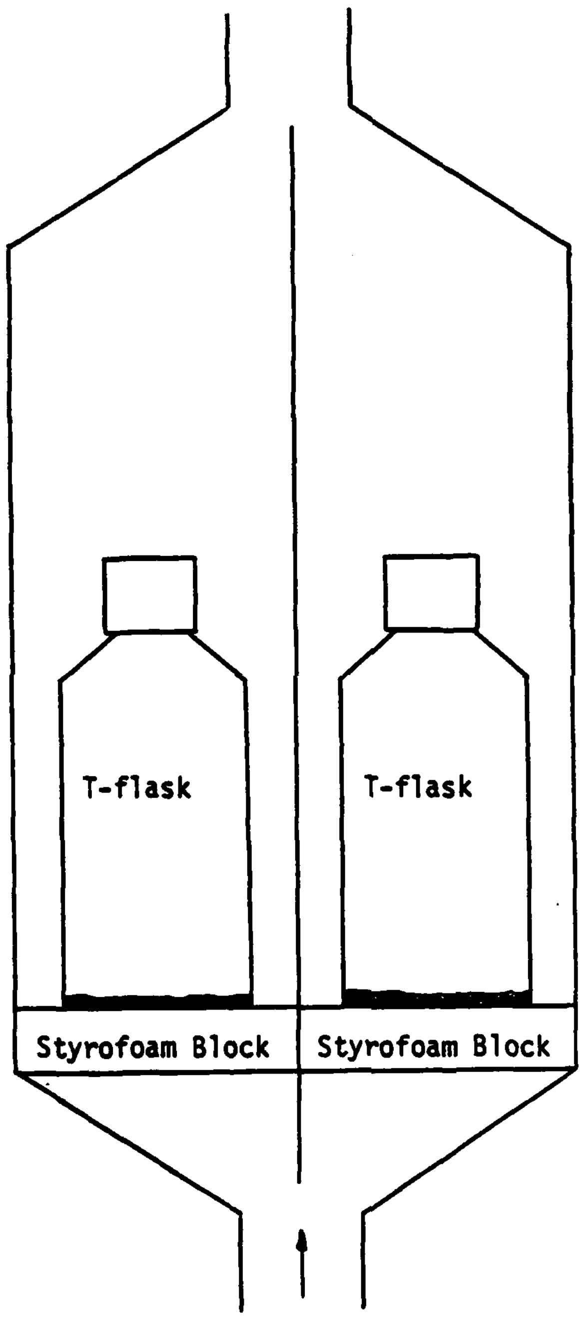

Figure 2. Geometric conformation of T-flasks in Crawford cell TEM test chamber during experiments with a power density of 25 mW/cm^2. The arrow denotes the direction of energy flow.

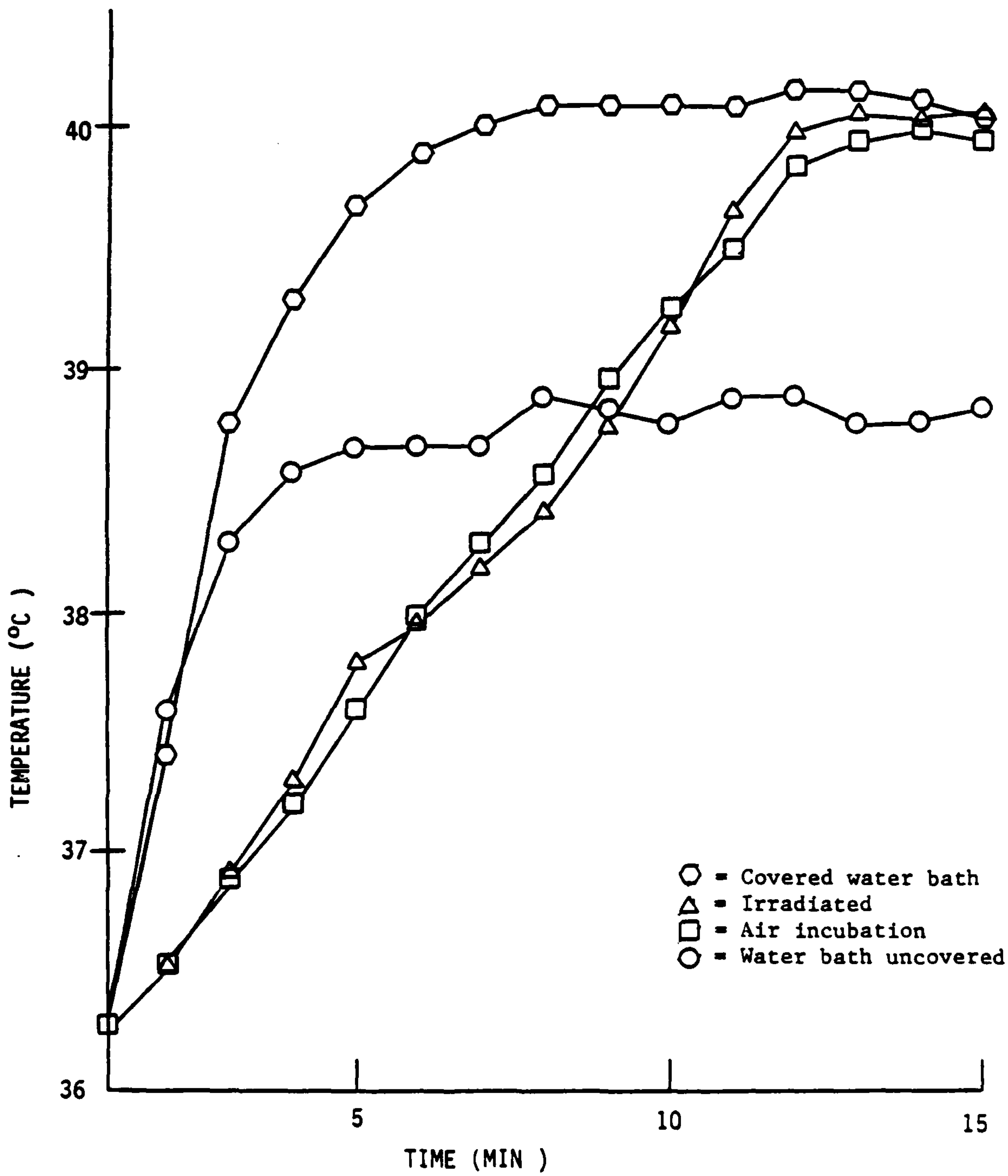

Figure 3. Temperature profiles of various heating methods.

Pulse-labelling and Processing of Anterior Pituitary Slices for Measurement of Release of Pulse-labelled Polypeptide Hormones

Anterior pituitary slices were pulse-labelled and processed as described for the pancreatic tissue slices, with these three exceptions:

(1) The cells were pulse-labelled in 3 ml medium I containing 25 μCi tritium-labelled L-amino acid mixture (New England Nuclear, Boston, Massachusetts),

(2) The 1.0- ml aliquots of chase incubation medium were collected after 60-, 120-, and 180- min postpulse incubation.

(3) Cells were stimulated to secrete by the addition of 20 μM norepinephrine.

Incubation Conditions for Anterior Pituitary Slices

The incubation conditions for anterior pituitary slices were the same as those for pancreatic tissue slices exposed to unmodulated 915-MHz radiation at a power density of 10 mW/cm^2.

Statistical Analysis

Data were analyzed using either a paired t-test or a Bonferroni t-test (2).

RESULTS

Our initial experiments with pancreatic tissue slices were conducted with slices exposed to incident power densities of 5 and 10 mW/cm^2. These power levels showed no effects on the secretion of pulse-labelled enzymes under either nonstimulated or carbamylcholine (CC)-stimulated conditions. Dosimetry experiments conducted at the United States Food and Drug Administration (USFDA) Bureau of Radiologic Health Laboratory (Rockville, Maryland) showed that at both power levels the SAR is less than 0.02 mW/g and there is no change of temperature of the medium bathing the tissue slices. Tables 1 and 2 show the results of 20 experiments conducted at 5 mW/cm^2, and Tables 3 and 4 show the results of 6 experiments conducted at 10 mW/cm^2.

All of our remaining experiments on pancreatic tissue slices were conducted with slices exposed to an incident power density of 25 mW/cm^2. Continuous-wave 915-MHz radiation at this power level has effects on secretion. Dosimetry measurements indicated that at this power level the SAR is 14.5 mW/g and there occurs a 3 °C increase in the temperature of the medium. Because of this temperature increase, all experiments conducted at a power density of 25 mW/cm^2 under either nonstimulated or CC-stimulated conditions were performed with three sets of tissue slices described in the Materials and Methods section.

Comparison of data acquired from the 37 °C control and 40 °C control sets of tissue slices in each experiment indicates any thermal effect on the secretory process when the tissue slices are KINETICALLY heated 3 °C above the normal incubation temperature. Comparison of data acquired from the 40 °C control and microwave-heated sets of tissue slices in each experiment indicates any difference in the secretory process between tissue slices KINETICALLY VS. ELECTROMAGNETICALLY heated 3 °C above the normal incubation temperature.

Table 5 shows that irradiation of nonstimulated tissue slices with 25 mW/cm^2 radiation increases amylase secretion by roughly 80% after 135- and 195- min exposure. By contrast, there is no difference in amylase secretion between tissue slices kinetically heated at 37 °C vs. 40 °C. This result is the most convincing piece of data we have acquired which shows that microwaves can alter the secretory process via nonthermal means. Table 6 shows that amylase secretion from CC-stimulated tissue slices is neither augmented nor inhibited by the microwave radiation; the cholinergic stimulation induces a 110% increase over the basal rate of amylase secretion. The data suggest that 915-MHz radiation increases zymogen granule discharge via the same intracellular mechanisms elicited by cholinergic stimulation.

Tables 7 and 8 show that the secretion of pulse-labelled enzymes from irradiated tissue slices is greater than that from slices incubated at 37 °C; this microwave stimulation is observed under both nonstimulated and CC-stimulated conditions. However, there are no significant differences in CUMULATIVE secretion between tissue slices kinetically vs. electromagnetically heated at 40 °C. The results thus suggest that the sum stimulatory effect that microwave exposure has on the last three major steps in the secretory process is equivalent to the sum stimulatory effect which can be produced by conventional kinetic means.

However, statistical analysis of the PERIODIC release of pulse-labelled enzymes under control conditions (Table 9) and CC-stimulated conditions (Table 10) shows that during the 90-150 min postpulse period under CC-stimulated conditions, there is a significant difference between tissue slices kinetically vs. electromagnetically heated to 40 °C; the rate of release during this period is 25% greater in the irradiated slices. This result prompted us to analyze by electron microscopic radioautography the distribution of pulse-labelled enzymes in CC-stimulated tissue slices 20 to 60 min after the pulse-label period (Tables 11-16). Silver grains were counted over rough endoplasmic reticulum (RER), peripheral vesicles of the Golgi complex (PV), condensing vacuoles (CV), zymogen granules (ZG), nuclei (N), mitochondria (M), and lysosomes (L).

In analyzing the radioautographic data in Tables 11-16, in the 20 min postpulse slices, the silver grains represent the intracellular distribution of 99.5% of the enzymes which were labelled during the pulse-label period. The silver grain distribution in 20 min postpulse slices is not significantly affected by any loss of secreted pulse-labelled enzymes. However, in the 60- min postpulse slices, the silver grains represent the intracellular distribution of 95-97% of the enzymes which were labelled during the pulse-label period. The silver grain distribution in 60 min postpulse slices is thus affected by as much as a 5% loss of the pulse-labelled enzymes.

Bonferroni analysis of the 20 min postpulse slices shows significant differences among the three sets of tissue slices in only the CV. In the 37 °C slices, 15% of the grains are found over CV; this figure is significantly lower in slices kinetically heated to 40 °C (6%) and in irradiated slices (9%). By contrast, statistical analysis of the 60 min postpulse slices shows significant differences among the three sets of tissue slices in only the ZG. In the slices kinetically heated to 40 °C, only 10% of the grains are found over ZG; this figure is significantly higher in 37 °C slices (28%) and in irradiated slices (30%). The most conservative conclusion which can be drawn from these analyses is that microwave radiation can alter the rates at which digestive enzymes are processed in condensing vacuoles and then accumulated in ZG. The data suggest that the microwaves are altering the kinetics of enzyme transport through these intracellular compartments by both thermal and nonthermal means. The altered kinetics of intracellular transport in irradiated slices during the first 60 min postpulse accounts for the increased secretion of the pulse-labelled enzymes during the 90-150 min postpulse period, as compared to 37 °C tissue slices and slices kinetically heated to 40 °C.

Ten separate experiments were also conducted to examine the effect of unmodulated 915-MHz radiation on the release of pulse-labelled polypeptide hormones from rat anterior pituitary tissue slices. Table 17 shows that the radiation, when applied at a power density of 10 mW/cm^2, has no statistically significant effect on either the nonstimulated or 20 μM norepinephrine-stimulated release of pulse-labelled hormones.

DISCUSSION

The studies with pancreatic tissue slices reported here show that 915-MHz radiation can alter the secretory process in the exocrine cells in two ways: (1) the radiation can alter the intracellular transport of digestive enzymes from the peripheral vesicles of the Golgi complex through condensing vacuoles and into zymogen granules, and (2) the radiation can increase exocytotic discharge of the contents of the zymogen granules.

Moreover, the studies with pancreatic tissue slices show that when microwaves alter exocrine secretory processes, the alterations do not occur by thermal means alone. In particular, the results show that microwaves increase the rate of ZG discharge by nonthermal means. This point was proven by heating 40 °C control flasks at the same rate as the irradiated flasks were heated to 40 °C. However, it is probably pertinent that microwave effects on pancreatic secretion were not observed unless the microwaves were applied a power density sufficient to cause heating of the pancreatic tissue. If microwaves can alter pancreatic secretion by field effects, then our data suggest that such field effects become evident only when microwave exposure is of such an intensity to also produce heating.

The use of T-flasks as in vitro incubation chambers enabled us to vary SAR at a given power density as a function of T-flask and culture medium orientation within the Crawford Cell TEM test chamber. We found that when the long axis of the T-flask and the thin film of culture medium are both oriented parallel to the center plate of the test chamber (i.e., the direction of microwave propagation) (Fig. 2), there occurs very little absorption of microwave energy. Under these conditions, the SAR is extremely low, even when the tissue slices are exposed to microwaves at the highest power density limits of our apparatus (about 27 mW/cm^2). By contrast, when the T-flasks and the thin film of culture medium are oriented as shown in Figure 3, there occurs enough energy absorption at 25 mW/cm^2 to raise the temperature of the medium by 3 °C.

Our exposure conditions were, therefore, not optimal from a geometrical point-of-view (i.e., from the viewpoint of the geometry which the tissue culture flasks presented to the microwaves). It would have been advantageous to have used cylindrically shaped culture chambers, and to have cultured the tissue slices in the absence of any contact with the plastic surfaces of the tissue culture flasks (so as to minimize the difference in the dielectric constants between the tissue slices and the media or surfaces with which they are in contact). However, we found that, when using tissue slices, it is difficult to introduce such modifications without also greatly restricting our ability to collect and replace media samples at specific time points. Our results show that the capacity of an experimental protocol to detect microwave effects on secretion depends to a significant extent upon the capacity to collect and replace media bathing the secretory tissue. Accordingly, our conditions for studying secretion from tissue slices were optimal from a procedural point-of-view.

We tried to study microwave effects on dispersed cell populations of anterior pituitary tissue, so we could use incubation chambers which present a suitable geometry in a well-defined microwave field. However, we were not able to reproduce published methods for culturing large numbers of dispersed anterior pituitary cells on Cytodex beads (8, 9). Microwave effects on secretion could be most easily conducted on dispersed secretory cells if derived cell populations were used.

We also found that when pancreatic tissue slices are heated to 40 $^{\circ}C$ by either kinetic or microwave means, there do not occur any alterations in pancreatic exocrine cell ultrastructure. The microwave effects on pancreatic secretion do not, therefore, arise from any observable effects on the structure or integrity of intracellular organelles. This observation is not surprising because core temperature in a human individual rises to 40 $^{\circ}C$ during moderate exercise.

CONCLUSION

The results of the in vitro studies with pancreatic tissue suggest that microwaves can alter exocrine and endocrine secretions in vivo under conditions where the microwaves increase body heat in general or in localized body regions. On the other hand, our results also suggest that if the microwaves are applied at power densities insufficient to produce "hot spots" in human and animal bodies, then no secretion effects can be detected.

REFERENCES

1. Bernfield, P. Amylases, α and β. Methods Enzymol 1:149-158 (1955).

2. Glantz, S.A. Primer of Biostatistics. New York: McGraw-Hill, N.Y. (1981).

3. Jamieson, J.D., and G.E. Palade. Intracellular Transport of Secretory Proteins in the Pancreatic Exocrine Cell I. Role of the Peripheral Elements of the Golgi Complex. J Cell Biol 34:577-596 (1967a).

4. Jamieson, J.D., and G.E. Palade. Intracellular Transport of Secretory Proteins in the Pancreatic Exocrine Cell II. Transport of Condensing Vacuoles and Zymogen Granules. J Cell Biol 34:597-615 (1967b).

5. Jamieson, J.D., and G.E. Palade. Synthesis, Intracellular Transport, and Discharge of Secretory Proteins in Stimulated Pancreatic Exocrine Cells. J Cell Biol 50:135-158 (1971a).

6. Jamieson, J.D., and G.E. Palade. Condensing Vacuole Conversion and Zymogen Granule Discharge in Pancreatic Exocrine Cells: Metabolic Studies. J Cell Biol 48:503-522 (1971b).

7. Nadler, N.J. Quantitation and Resolution in Electron Microscope Radioautography. J Histochem Cytochem 27:1531-1533 (1979).

8. Smith, M.A., and W.W. Vale. Superfusion of Rat Anterior Pituitary Cells Attached to Cytodex Beads: Validation of a Technique. Endocrinology 107:1425-1431 (1980).

9. Smith, M.A., and W.W. Vale. Desensitization to Gonadotropin-Releasing Hormone Observed in Superficial Pituitary Cells on Cytodex Beads. Endocrinology 108:752-759 (1981).

TABLE 1. THE RELEASE OF PULSE-LABELLED SECRETORY PROTEINS FROM NONSTIMULATED PANCREATIC TISSUE SLICES INCUBATED AT 37 °C OR IRRADIATED WITH 915-MHz RADIATION, POWER DENSITY 5 mW/cm^2 (SAR < 0.02 mW/g)

	30 min		90 min		150 min		210 min	
Expt #	37	I	37	I	37	I	37	I
1	0	0.1	1.5	0.8	1.2	1.3	2.6	1.6
2	0	0	0.5	1.6	1.9	4.2	2.9	5.4
3	1.1	0	1.3	1.8	2.8	3.6	3.6	3.7
4	1.0	0	1.2	1.6	1.7	2.3	2.4	3.2
5	0.4	0	0.7	0.9	1.2	1.8	2.3	2.6
6	0	0	1.4	0.8	2.3	2.0	3.3	2.6
7	0	0	1.0	0.8	2.8	2.2	5.2	3.8
8	0	0	3.2	0.8	4.8	2.3	6.9	2.8
9	0	0	1.0	1.0	2.1	1.9	2.3	3.4
10	0	0	0.8	0.8	1.8	1.6	3.2	2.1
11	0	0	0.7	1.1	1.7	2.4	2.8	3.1
12	0	0	0.7	0.9	1.6	1.8	1.8	2.3
13	0.3	0	1.1	0.8	2.0	1.7	2.9	2.4
14	0	0	0.6	0.6	1.7	2.4	2.5	2.7
15	1.0	0.3	1.1	1.3	2.4	1.9	3.0	2.5
16	1.0	0.6	1.6	1.6	2.8	3.0	3.5	4.5
17	0.4	0	1.2	1.1	2.8	2.9	2.8	2.4
18	0.9	1.7	1.6	2.4	3.3	3.6	4.2	4.5
19	0.4	1.0	1.3	1.4	2.0	2.3	3.1	3.1
20	0.8	1.4	1.5	1.9	3.2	2.9	3.8	4.2

	30 min	90 min	150 min	210 min
Mean Difference	0.11	0.00	-0.10	0.11
SD	0.48	0.71	0.89	1.29
t	1.02	0.00	-0.50	0.38
df	19	19	19	19

TABLE 2. THE RELEASE OF PULSE-LABELLED SECRETORY PROTEINS FROM CARBAMYLCHOLINE-STIMULATED PANCREATIC TISSUE SLICES INCUBATED AT 37 °C OR IRRADIATED WITH 915-MHz RADIATION, POWER DENSITY 5 mW/cm^2 (SAR < 0.02 mW/g)

	30 min		90 min		150 min		210 min	
Expt #	CC37	CCI	CC37	CCI	CC37	CCI	CC37	CCI
1	0.3	0	6.5	5.9	20.0	21.3	31.8	29.7
2	0	0	8.5	6.2	19.8	22.1	24.8	26.0
3	0	0	9.0	7.9	26.3	21.2	33.8	24.6
4	0	0	13.4	11.1	27.3	23.7	34.7	27.5
5	0	0	3.7	3.7	13.1	14.5	31.2	31.2
6	0	0	5.1	5.7	17.8	17.6	29.6	26.6
7	0	0	3.7	3.9	15.0	16.8	24.5	27.6
8	0	0.3	2.9	3.3	13.9	14.3	23.2	21.4
9	0	0.4	0.7	4.9	19.2	11.4	31.1	20.3
10	0	0	5.2	0.7	15.3	19.8	20.8	28.5
11	0	0	4.6	0.5	18.0	18.2	30.8	36.3
12	0	0	4.4	4.1	17.3	21.9	29.3	34.8
13	0	0	5.4	3.7	21.2	16.6	34.3	28.5
14	0	0	3.7	3.4	14.2	13.3	24.0	23.6
15	0.8	1.0	6.9	7.7	20.6	24.7	32.0	34.8
16	0.3	0.5	4.5	4.8	15.0	15.9	24.5	25.7
17	1.4	0.8	1.7	3.8	13.3	14.3	23.3	23.3
18	0.9	0.5	9.0	7.5	28.5	26.3	39.8	34.1
19	0.5	1.1	5.2	5.7	20.1	23.3	40.7	37.9
20	0.6	0.7	7.2	5.5	21.8	19.2	31.0	30.0
Mean Difference		-0.03		0.57		0.07		1.14
SD		0.26		1.98		3.37		4.92
t		-0.43		1.27		0.09		1.04
df		19		19		19		19

TABLE 3. THE RELEASE OF PULSE-LABELLED SECRETORY PROTEINS FROM NONSTIMULATED PANCREATIC TISSUE SLICES INCUBATED AT 37 °C OR IRRADIATED WITH 915-MHz RADIATION, POWER DENSITY 10 mW/cm^2 (SAR < 0.02 mW/g)

30-min Incubation

Expt #	37	I
1	0	0
2	0	0
3	0	0
4	0.2	0
5	0	0
6	0	0
Mean Difference		0.03
SD		0.08
t		1.00
df		5

90-min Incubation

Expt #	37	I
1	1.6	1.1
2	2.5	1.2
3	1.7	1.9
4	0.5	0.9
5	0.9	1.4
6	0.5	0
Mean Difference		0.20
SD		0.69
t		0.71
df		5

150-min Incubation

Expt #	37	I
1	2.3	2.3
2	2.8	2.1
3	4.1	4.3
4	2.1	2.1
5	2.2	2.6
6	2.3	1.6
Mean Difference		0.13
SD		0.46
t		0.70
df		5

210-min Incubation

Expt #	37	I
1	4.2	4.1
2	6.3	2.9
3	6.2	5.3
4	3.1	3.3
5	2.7	3.0
6	2.0	2.1
Mean Difference		0.63
SD		1.42
t		1.09
df		5

TABLE 4. THE RELEASE OF PULSE-LABELLED SECRETORY PROTEINS FROM CARBAMYLCHOLINE-STIMULATED PANCREATIC TISSUE SLICES INCUBATED AT 37 °C OR IRRADIATED WITH 915-MHz RADIATION, POWER DENSITY 10 mW/cm^2 (SAR < 0.02 mW/g)

30-min Incubation

Expt #	CC37	CCI
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	0	0
Mean Difference		0
SD		0
t		0
df		5

90-min Incubation

Expt #	CC37	CCI
1	6.2	4.5
2	8.1	7.3
3	8.6	22.2
4	6.5	6.7
5	8.0	11.1
6	6.1	3.9
Mean Difference		-2.03
SD		5.97
t		-0.83
df		5

150-min Incubation

Expt #	CC37	CCI
1	22.2	20.6
2	17.2	17.9
3	29.4	32.3
4	18.8	24.7
5	25.3	28.8
6	29.9	20.8
Mean Difference		-0.38
SD		5.30
t		-0.18
df		5

210-min Incubation

Expt #	CC37	CCI
1	31.0	35.7
2	19.7	21.6
3	41.7	42.1
4	33.0	35.1
5	33.6	37.3
6	44.0	34.9
Mean Difference		-0.62
SD		4.99
t		-0.30
df		5

TABLE 5. RELEASE OF STORED AMYLASE FROM NONSTIMULATED TISSUE IRRADIATED WITH AN SAR OF 14.5 mW/g

15-min Incubation

Expt #	37	I	40
1	2.5	4.2	3
2	2	3	2
3	2.7	3.5	2.8
4	2	3.6	1.7
Mean	2.3	3.58	2.38
STD	0.31	0.43	0.54
F=	22.52		

Bonferroni analysis:
p<0.05=3.370
37/40=0.374
37/I =5.991
40/I =5.616

75-min Incubation

Expt #	37	I	40
1	4.6	5.8	6.2
2	4.3	5.2	3.1
3	4.3	3.8	3.9
4	2.3	4.8	2.6
Mean	3.88	4.9	3.95
STD	0.92	0.73	1.38
F=	1.597		

Bonferroni analysis:
p<0.05=3.370
37/40=0.11
37/I =1.6
40/I =1.49

135-min Incubation

Expt #	37	I	40
1	5.3	10.6	6.5
2	4.9	8.3	5.5
3	3.9	5.6	4.6
4	4	10.8	2.8
Mean	4.525	8.83	4.85
STD	0.59	2.11	1.36
F=	9.345		

Bonferroni analysis:
p<0.05=3.370
37/40=0.293
37/I =3.882
40/I =3.589

195-min Incubation

Expt #	37	I	40
1	11.3	16.3	10.7
2	7.8	11.5	7.4
3	7.2	16.3	9.9
4	6.3	14.5	4.4
Mean	8.15	14.65	8.1
STD	1.9	1.96	2.46
F=	20.15		

Bonferroni analysis:
p<0.05=3.370
37/40=0.042
37/I =5.477
40/I =5.519

TABLE 6. RELEASE OF STORED AMYLASE FROM CARBAMLYCHOLINE-STIMULATED TISSUE IRRADIATED WITH AN SAR OF 14.5 mW/g

15-min Incubation

Expt #	CC37	CCI	CC40
1	4.4	2.5	3.4
2	5.5	4.2	4.9
3	3.2	4.9	4.5
4	2.6	4.4	5.1
Mean	3.925	4	4.48
STD	1.12	0.9	0.66
F=	0.318		

Bonferroni analysis:
p<0.05=3.370
37/40=0.735
37/I =0.099
40/I =0.636

75-min Incubation

Expt #	CC37	CCI	CC40
1	5	6	4.7
2	11.5	8.9	10.1
3	3.8	6	5.5
4	3.3	11.3	11.3
Mean	5.9	8.05	7.9
STD	3.29	2.22	2.85
F=	0.907		

Bonferroni analysis:
p<0.05=3.370
37/40=1.122
37/I =1.206
40/I =0.084

135-min Incubation

Expt #	CC37	CCI	CC40
1	8.5	10.6	7.7
2	17.9	13.5	22.7
3	4.7	10.9	8.9
4	7.6	16.7	15.7
Mean	9.675	12.93	13.75
STD	4.95	2.45	6
F=	1.402		

Bonferroni analysis:
p<0.05=3.370
37/40=1.583
37/I =1.264
40/I =0.319

195-min Incubation

Expt #	CC37	CCI	CC40
1	14.7	8.2	9.2
2	26.4	25.9	29
3	17.5	21.6	19.5
4	24.6	27.4	23.5
Mean	20.8	20.78	20.3
STD	4.85	7.57	7.24
F=	0.041		

Bonferroni analysis:
p<0.05=3.370
37/40=0.256
37/I =0.01
40/I =0.245

TABLE 7. RELEASE OF PULSE-LABELLED SECRETORY PROTEINS FROM NONSTIMULATED TISSUE IRRADIATED WITH AN SAR OF 14.5 mW/g

30-min Incubation			
Expt #	37	I	40
1	0.3	0.5	0.4
2	0.3	0.3	0.4
3	0.2	0.4	0.4
4	0.2	0.2	0.3
5	0.5	0.3	0.5
6	0.3	0.2	0.3
7	0.3	0.2	0.3
8	0.4	0.2	0.7
9	0.3	0.2	0.2
10	0.3	0.2	0.7
Mean	0.31	0.27	0.42
STD	0.08	0.1	0.16
F=	4.270		

Bonferroni analysis:
p<0.05=2.682
37/40=2.067
37/I =0.751
40/I =2.818

90-min Incubation			
Expt #	37	I	40
1	1.7	2.5	1.8
2	1.5	2.1	1.6
3	0.9	1.7	1.5
4	1	1.4	1.9
5	1.4	1.6	1.6
6	1.3	2.2	1.9
7	1.2	1.5	1.3
8	1.4	1.5	2.1
9	1.2	1.5	1.6
10	1.8	2	2.3
Mean	1.34	1.8	1.76
STD	0.27	0.35	0.28
F=	11.01		

Bonferroni analysis:
p<0.05=2.682
37/40=3.868
37/I =4.237
40/I =0.368

150-min Incubation			
Expt #	37	I	40
1	2.7	4.3	3.1
2	2.8	4.1	3
3	2.4	3.8	3.4
4	2.4	2.8	3.4
5	3	4	4.8
6	3	4.7	5.3
7	2.5	2.8	2.6
8	3.1	3	4.1
9	2.5	3.7	4.5
10	3.2	2.6	5.2
Mean	2.76	3.58	3.94
STD	0.29	0.69	0.92
F=	8.645		

Bonferroni analysis:
p<0.05=2.682
37/40=4.057
37/I =2.819
40/I =1.238

210-min Incubation			
Expt #	37	I	40
1	3.9	4.9	5
2	3.7	5.1	4.5
3	3.5	4.9	5
4	3.6	4.9	4.8
5	5.8	6.4	8.4
6	4.7	7.1	8.4
7	4.1	4.3	4.4
8	5	6.1	7.4
9	6.9	6.8	9.2
10	5.6	7.4	8.8
Mean	4.68	5.79	6.59
STD	1.08	1.04	1.91
F=	19.88		

Bonferroni analysis:
p<0.05=2.682
37/40=6.279
37/I =3.649
40/I =2.63

TABLE 8. THE RELEASE OF PULSE-LABELLED SECRETORY PROTEINS FROM CARBAMYLCHOLINE-STIMULATED PANCREATIC TISSUE SLICES INCUBATED AT 37 °C, AT 40 °C, OR IRRADIATED WITH 915-MHz RADIATION. POWER DENSITY 25 mW/cm^2 (SAR = 14.5 mW/g)

30-min Incubation

Expt #	CC37	CCI	CC40
1	0.3	0.4	0.7
2	0.3	0.9	0.6
3	0.3	0.4	0.6
4	0.2	0.3	0.7
5	0.4	0.3	0.5
6	0.2	0.4	0.5
7	0.4	0.2	0.4
8	0.2	0.5	0.2
9	0.4	0.2	0.3
10	0.2	0.2	0.4
Mean	0.29	0.38	0.49
STD	0.08	0.2	0.16
F=	3.984		

Bonferroni analysis:
p<0.05=2.682
37/40=2.816
37/I =1.267
40/I =1.549

90-min Incubation

Expt #	CC37	CCI	CC40
1	5.2	11.2	11.0
2	4.6	6.9	9.8
3	4.0	14.5	12.8
4	11.9	8.6	14.3
5	3.9	6.6	7.1
6	4.1	8.3	7.8
7	4.4	10.3	14.2
8	5.6	9.1	6.9
9	7.6	10.5	13.6
10	5.3	9.6	10.9
Mean	5.66	9.56	10.84
STD	2.32	2.17	2.73
F=	16.86		

Bonferroni analysis:
p<0.05=2.682
37/40=5.574
37/I =4.197
40/I =1.377

150-min Incubation

Expt #	CC37	CCI	CC40
1	18.5	32.0	26.1
2	18.1	21.9	22.5
3	18.5	38.6	26.7
4	26.2	29.3	28.9
5	16.2	23.7	22.4
6	16.2	21.9	22.3
7	22.3	30.3	30.2
8	21.5	24.4	17.2
9	23.9	22.8	28.7
10	20.4	27.5	24.8
Mean	20.18	27.24	24.98
STD	3.13	5.13	3.76
F=	10.52		

Bonferroni analysis:
p<0.05=2.682
37/40=3.055
37/I =4.493
40/I =1.438

210-min Incubation

Expt #	CC37	CCI	CC40
1	31.0	39.7	31.3
2	26.4	29.4	26.2
3	30.1	51.9	35.0
4	42.8	45.7	39.6
5	29.1	31.0	32.9
6	30.4	35.5	37.1
7	36.3	41.8	40.3
8	35.5	33.5	25.1
9	35.0	33.3	37.6
10	32.6	38.3	34.2
Mean	32.92	38.01	33.93
STD	4.42	6.64	4.93
F=	4.014		

Bonferroni analysis:
p<0.05=2.682
37/40=0.531
37/I =2.676
40/I =2.145

TABLE 9. THE RATE OF RELEASE OF PULSE-LABELLED SECRETORY PROTEINS FROM NONSTIMULATED PANCREATIC TISSUE SLICES INCUBATED AT 37 °C, OR IRRADIATED WITH 915-MHz RADIATION. POWER DENSITY 25 mW/cm^2 (SAR=14.5 mW/g)

0-30 min

Expt #	37	I	40
1	0.3	0.5	0.4
2	0.3	0.3	0.4
3	0.2	0.4	0.4
4	0.2	0.2	0.3
5	0.5	0.3	0.5
6	0.3	0.2	0.3
7	0.3	0.2	0.3
8	0.4	0.2	0.7
9	0.3	0.2	0.2
10	0.3	0.2	0.7
Mean	0.31	0.27	0.42
STD	0.08	0.1	0.16
F=	4.270		

Bonferroni analysis:
p<0.05=2.682
37/40=2.067
37/I =0.751
40/I =2.818

30-90 min

Expt #	37	I	40
1	1.4	2	1.4
2	1.2	1.8	1.2
3	0.7	1.3	1.1
4	0.8	1.2	1.6
5	0.9	1.3	1.1
6	1	2	1.6
7	0.9	1.3	1
8	1	1.3	1.4
9	0.9	1.3	1.4
10	1.5	1.8	1.6
Mean	1.03	1.53	1.34
STD	0.25	0.31	0.22
F=	16.96		

Bonferroni analysis:
p<0.05=2.682
37/40=3.577
37/I =5.769
40/I =2.192

90-150 min

Expt #	37	I	40
1	1	1.8	1.3
2	1.3	2	1.4
3	1.5	2.1	1.9
4	1.4	1.4	1.5
5	1.6	2.4	3.2
6	1.7	2.5	3.4
7	1.3	1.3	1.3
8	1.7	1.5	2
9	1.3	2.2	2.9
10	1.4	0.6	2.9
Mean	1.42	1.78	2.18
STD	0.2	0.55	0.79
F=	5.410		

Bonferroni analysis:
p<0.05=2.682
37/40=3.288
37/I =1.557
40/I =1.73

150-210 min

Expt #	37	I	40
1	1.2	0.6	1.9
2	0.9	1	1.5
3	1.1	1.1	1.6
4	1.2	2.1	1.4
5	2.8	2.4	3.6
6	1.7	2.4	3.1
7	1.6	1.5	1.8
8	1.9	3.1	3.3
9	4.4	3.1	4.7
10	2.4	4.8	3.6
Mean	1.92	2.21	2.65
STD	1	1.19	1.09
F=	3.898		

Bonferroni analysis:
p<0.05=2.682
37/40=2.773
37/I =1.102
40/I =1.671

TABLE 10. THE RATE OF RELEASE OF PULSE-LABELLED SECRETORY PROTEINS FROM CARBAMYLCHOLINE-STIMULATED PANCREATIC TISSUE SLICES INCUBATED AT 37 °C, AT 40 °C, OR IRRADIATED WITH 915-MHz RADIATION, POWER DENSITY 25 mW/cm^2 (SAR = 14.5 mW/g)

0-30 min				30-90 min			
Expt #	CC37	CCI	CC40	Expt #	CC37	CCI	CC40
1	0.3	0.4	0.7	1	4.9	10.8	10.3
2	0.3	0.9	0.6	2	4.3	6.0	9.2
3	0.3	0.4	0.6	3	3.7	14.1	12.2
4	0.2	0.3	0.7	4	11.7	8.3	13.6
5	0.4	0.3	0.5	5	3.5	6.3	6.6
6	0.2	0.4	0.5	6	3.9	7.9	7.3
7	0.4	0.2	0.4	7	4.0	10.1	13.8
8	0.2	0.5	0.2	8	5.4	8.6	6.7
9	0.4	0.2	0.3	9	7.2	10.3	13.3
10	0.2	0.2	0.4	10	5.1	9.4	10.9
Mean	0.29	0.38	0.49	Mean	5.37	9.18	10.39
STD	0.08	0.2	0.16	STD	2.35	2.24	2.7
F=	3.984			F=	15.76		

Bonferroni analysis (0-30 min):
$p<0.05$=2.682
37/40=2.816
37/I =1.267
40/I =1.549

Bonferroni analysis (30-90 min):
$p<0.05$=2.682
37/40=5.381
37/I =4.084
40/I =1.297

90-150 min				150-210 min			
Expt #	CC37	CCI	CC40	Expt #	CC37	CCI	CC40
1	13.3	20.8	15.1	1	12.5	7.7	5.2
2	13.5	15.0	12.7	2	8.3	7.5	3.7
3	14.5	24.1	13.9	3	11.6	13.3	8.3
4	14.3	20.7	14.6	4	16.6	16.4	10.7
5	12.3	17.1	15.3	5	12.9	7.3	10.5
6	12.1	13.6	14.5	6	14.2	13.6	14.8
7	17.9	20.0	16.0	7	14.0	11.5	10.1
8	15.9	15.3	10.3	8	14.0	9.1	7.9
9	16.3	12.3	15.1	9	11.1	10.5	8.9
10	15.1	17.9	13.9	10	12.2	10.8	9.4
Mean	14.52	17.68	14.14	Mean	12.74	10.77	8.95
STD	1.74	3.53	1.55	STD	2.1	2.86	2.9
F=	6.298			F=	11.79		

Bonferroni analysis (90-150 min):
$p<0.05$=2.682
37/40=3.347
37/I =2.885
40/I =3.232

Bonferroni analysis (150-210 min):
$p<0.05$=2.682
37/40=4.855
37/I =2.523
40/I =2.331

TABLE 11. LOCALIZATION OF SILVER GRAINS OVER ORGANELLES IN CARBAMYLCHOLINE-STIMULATED CELLS INCUBATED FOR 20 MIN AT 37 °C, AT 40 °C, OR IRRADIATED WITH 25 mW/cm^2 (SAR = 14.5 mW/g)

Expt #	RER	PV	CV	ZG	N	M	L	Total
			Grains Counted Over Tissue Incubated at 37 °C					
1	205	94	86	21	8	23	0	437
2	527	233	170	102	31	38	1	1102
3	473	165	81	57	10	25	2	813
4	385	437	163	137	11	27	0	1160
Percentages:								
1	47	22	20	5	2	5	0	101
2	48	21	15	9	3	3	0	99
3	58	20	10	7	1	3	0	99
4	33	38	14	12	1	2	0	100
Mean:	47	25	15	8	2	3	0	
			Grains Counted Over Irradiated Tissue					
1	324	137	107	15	12	17	1	613
2	401	104	50	36	20	37	0	648
3	368	45	3	46	13	20	4	499
4	244	113	46	34	13	8	0	458
Percentages:								
1	53	22	17	2	2	3	0	99
2	62	16	8	6	3	6	0	101
3	74	9	1	9	3	4	1	101
4	53	25	10	7	3	2	0	100
Mean:	61	18	9	6	3	4	0	
			Grains Counted Over Cells Incubated at 40 °C					
1	875	389	184	106	28	50	4	1636
2	284	53	19	50	10	24	3	443
3	153	66	12	28	9	18	0	286
4	693	91	26	34	6	48	2	900
Percentages:								
1	53	24	11	6	2	3	0	99
2	64	12	4	11	2	5	1	99
3	53	23	4	10	3	6	0	99
4	77	10	3	4	1	5	0	100
Mean:	62	17	6	8	2	5	0	

TABLE 12. ANALYSIS OF SILVER GRAIN LOCALIZATION AFTER 20 MIN OF INCUBATION (FROM TABLE 11)

Rough endoplasmic reticulum				Peripheral vesicles			
Expt #	CC37	CCI	CC40	Expt #	CC37	CCI	CC40
1	47	53	53	1	22	22	24
2	48	62	64	2	21	16	12
3	58	74	53	3	20	9	23
4	33	53	77	4	38	25	10
Mean	46.5	60.5	61.75	Mean	25.25	18.0	17.25
STD	8.9	8.62	9.88	STD	7.4	6.12	6.3
F=	2.114			F=	1.205		

Bonferroni analysis (Rough endoplasmic reticulum):
$p<0.05=3.370$
37/40=1.852
37/I =1.7
40/I =0.152

Bonferroni analysis (Peripheral vesicles):
$p<0.05=3.370$
37/40=1.406
37/I =1.274
40/I =0.132

Condensing vacuoles				Zymogen granules			
Expt #	CC37	CCI	CC40	Expt #	CC37	CCI	CC40
1	20	17	11	1	5	2	6
2	15	8	4	2	9	6	11
3	10	1	4	3	7	9	10
4	14	10	3	4	12	7	4
Mean	14.75	9	5.5	Mean	8.25	6	7.75
STD	3.56	5.7	3.2	STD	2.59	2.55	2.86
F=	15.62			F=	0.705		

Bonferroni analysis (Condensing vacuoles):
$p<0.05=3.370$
37/40=5.536
37/I =3.441
40/I =2.095

Bonferroni analysis (Zymogen granules):
$p<0.05=3.370$
37/40=0.251
37/I =1.131
40/I =0.879

TABLE 13. ANALYSIS OF SILVER GRAIN LOCALIZATION AFTER 20 MIN OF INCUBATION (FROM TABLE 11)

Nucleus				Mitochondria			
Expt #	CC37	CCI	CC40	Expt #	CC37	CCI	CC40
1	2	2	2	1	5	3	3
2	3	3	2	2	3	6	5
3	1	3	3	3	3	4	6
4	1	3	1	4	2	2	5
Mean	1.75	2.75	2	Mean	3.25	3.75	4.75
STD	0.83	0.43	0.71	STD	1.09	1.48	1.09
F=	1.695			F=	1.049		

Nucleus — Bonferroni analysis:
p<0.05=3.370
37/40=0.442
37/I =1.769
40/I =1.327

Mitochondria — Bonferroni analysis:
p<0.05=3.370
37/40=1.423
37/I =0.474
40/I =0.949

Lysosomes			
Expt #	CC37	CCI	CC40
1	0	0	0
2	0	0	1
3	0	1	0
4	0	0	0
Mean	0	0.25	0.25
STD	0	0.43	0.43
F=	0.427		

Bonferroni analysis:
p<0.05=3.370
37/40=0.8
37/I =0.8
40/I = 0

TABLE 14. LOCALIZATION OF SILVER GRAINS OVER ORGANELLES IN CARBAMYLCHOLINE-STIMULATED CELLS INCUBATED FOR 60 MIN AT 37 °C, AT 40 °C, OR IRRADIATED WITH 25 mW/cm^2 (SAR 14.5 mW/g)

Expt #	RER	PV	CV	ZG	N	M	L	Total
			Grains Counted Over Cells Incubated at 37 °C					
1	220	166	75	154	28	27	1	671
2	122	156	79	137	10	19	2	525
3	250	192	64	283	34	31	1	855
4	104	212	69	181	8	9	0	583
Percentages:								
1	33	25	11	23	4	4	0	100
2	23	30	15	26	2	4	0	100
3	29	22	7	33	4	4	0	99
4	18	36	12	31	1	2	0	100
Mean:	26	28	11	28	3	4	0	
			Grains Counted Over Irradiated Cells					
1	95	105	48	115	19	15	6	403
2	328	143	41	242	24	18	9	805
3	281	67	45	267	2	6	0	668
4	361	290	197	220	46	46	0	1160
Percentages:								
1	24	26	12	29	5	4	1	101
2	41	18	5	30	3	2	1	100
3	42	10	7	40	0	1	0	100
4	31	25	17	19	4	4	0	100
Mean:	35	20	10	30	3	3	1	
			Grains Counted Over Cells Incubated at 40 °C					
1	464	125	75	154	7	26	3	854
2	252	150	76	32	10	15	0	535
3	184	125	26	45	37	58	15	490
4	484	286	267	79	6	48	0	1170
Percentages:								
1	54	15	9	18	1	3	0	100
2	47	28	14	6	2	3	0	100
3	38	26	5	7	8	12	3	99
4	41	24	23	7	1	4	0	100
Mean:	45	23	13	10	3	6	1	

TABLE 15. ANALYSIS OF SILVER GRAIN LOCALIZATION AFTER 60 MIN OF INCUBATION (FROM TABLE 14)

Rough endoplasmic reticulum			
Expt #	CC37	CCI	CC40
1	33	24	54
2	23	41	47
3	29	42	38
4	18	31	41
Mean	25.75	34.5	45
STD	5.72	7.43	6.12
F=	5.608		

Bonferroni analysis:
p<0.05=3.370
37/40=3.345
37/I =1.52
40/I =1.824

Peripheral vesicles			
Expt #	CC37	CCI	CC40
1	25	26	15
2	30	18	28
3	22	10	26
4	36	25	24
Mean	28.25	19.75	23.25
STD	5.31	6.42	4.97
F=	1.840		

Bonferroni analysis:
p<0.05=3.370
37/40=1.123
37/I =1.909
40/I =0.786

Condensing vacuoles			
Expt #	CC37	CCI	CC40
1	11	12	9
2	15	5	14
3	7	7	5
4	12	17	23
Mean	11.25	10.25	12.75
STD	2.86	4.66	6.72
F=	0.327		

Bonferroni analysis:
p<0.05=3.370
37/40=0.482
37/I =0.322
40/I =0.804

Zymogen granules			
Expt #	CC37	CCI	CC40
1	23	29	18
2	26	30	6
3	33	40	7
4	31	19	7
Mean	28.25	29.5	9.5
STD	3.96	7.43	4.92
F=	10.76		

Bonferroni analysis:
p<0.05=3.370
37/40=3.883
37/I =0.259
40/I =4.142

TABLE 16. ANALYSIS OF SILVER GRAIN LOCALIZATION AFTER 60 MIN OF INCUBATION (FROM TABLE 14)

Nucleus				Mitochondria			
Expt #	CC37	CCI	CC40	Expt #	CC37	CCI	CC40
1	4	5	1	1	4	4	3
2	2	3	2	2	4	2	3
3	4	0	8	3	4	1	12
4	1	4	1	4	2	4	4
Mean	2.75	3	3	Mean	3.5	2.75	5.5
STD	1.3	1.87	2.92	STD	0.87	1.3	3.77
F=	0.010			F=	0.900		

Nucleus — Bonferroni analysis:
p<0.05=3.370
37/40=0.126
37/I =0.126
40/I = 0

Mitochondria — Bonferroni analysis:
p<0.05=3.370
37/40=0.944
37/I =0.354
40/I =1.298

Lysosomes			
Expt #	CC37	CCI	CC40
1	0	1	0
2	0	1	0
3	0	0	3
4	0	0	0
Mean	0	0.05	0.75
STD	0	0.05	1.3
F=	0.567		

Bonferroni analysis:
p<0.05=3.370
37/40=1.046
37/I =0.697
40/I =0.349

TABLE 17. CUMULATIVE PERCENT RELEASE OF TOTAL PULSE-LABELLED SECRETORY PROTEINS FROM ANTERIOR PITUITARY CULTURES IRRADIATED AT 915-MHz (CW) WITH A POWER DENSITY OF 10 mW/cm^2

	Nonstimulated				Stimulated (20 µm norepinephrine)			
Expt #	Control		Irradiated		Control		Irradiated	
	1-h incubation				1-h incubation			
1	11.6		14.6		11.6		8.2	
2	9.3		6.9		6.2		10.6	
3	6.3		7.3		7.1		7.9	
4	7.3		6.9		7.8		10.2	
5	7.7		8.0		7.3		9.9	
6	8.3		8.7		11.0		7.9	
7	11.0		9.2		8.5		10.1	
8	11.4		7.8		8.2		7.4	
9	12.1		8.9		4.8		7.3	
10	13.1		12.5		15.5		8.7	
Mean and SD	9.9	2.2	9.1	2.5	8.8	3.1	8.8	1.3
	2-h incubation				2-h incubation			
1	16.9		19.4		14.5		11.7	
2	12.2		11.3		9.9		11.9	
3	11.7		12.0		9.0		10.5	
4	8.4		8.7		9.5		12.0	
5	11.8		8.2		8.3		12.2	
6	9.2		11.7		14.9		15.2	
7	15.4		11.7		10.8		13.0	
8	10.6		9.6		10.6		8.5	
9	16.4		14.3		8.2		11.4	
10	20.7		23.4		21.3		24.0	
Mean and SD	13.3	3.9	13.0	4.8	11.7	4.1	13.0	4.2
	3-h incubation				3-h incubation			
1	28.3		23.0		33.6		25.1	
2	16.5		12.8		11.1		12.9	
3	14.6		11.5		10.2		11.7	
4	10.3		8.7		9.4		12.6	
5	14.0		14.4		10.4		16.1	
6	11.8		10.7		16.3		16.6	
7	17.5		13.9		13.0		15.0	
8	9.9		10.9		12.9		9.1	
9	20.9		18.4		9.5		14.1	
10	25.5		27.5		26.6		28.0	
Mean and SD	16.9	6.3	15.2	6.0	15.3	8.2	16.1	6.0

END
11-87
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